



Apparent digestibility of nutrients and energy in extruded diets from cultivars of barley and wheat selected for nutritional quality in rainbow trout *Oncorhynchus mykiss*

T.G. GAYLORD¹, F.T. BARROWS¹, S.D. RAWLES², K. LIU³, P. BREGITZER³, A. HANG³, D.E. OBERT³ & C. MORRIS⁴

¹ USDA/ARS/PWA, Small Grains and Potato Germplasm Research Unit, Hagerman Fish Culture Experiment Station, Hagerman, ID, USA; ² USDA/ARS/SPA, Harry K. Dupree Stuttgart National Aquaculture Research Center, Stuttgart, AR, USA; ³ USDA/ARS/PWA, Small Grains and Potato Germplasm Research Unit, Aberdeen, ID, USA; ⁴ USDA/ARS/PWA, USDA/ARS Western Wheat Quality Laboratory, Food Science & Human Nutrition Facility East, Washington State University, Pullman, WA, USA

Abstract

A digestibility trial was conducted to elucidate potential differences in barley (*Hordeum vulgare* L.) lines and varieties as a first step in defining their potential for use in aquafeeds. A diverse group of six barley lines having six combinations of selected attributes, normal versus low phytic acid, normal versus waxy, and hulled versus hull-less, were chosen for evaluation along with a waxy wheat (*Triticum aestivum* L.) and soft white wheat. The experimental diets were manufactured by cooking extrusion and consisted of a 70% reference diet that was formulated to contain a minimal level of phosphorus and 30% test ingredient. Phosphorus availability ranged from 17 to 78% and was influenced by starch type in wheat. Apparent protein digestibility ranged from 53 to 125% and differences were observed between wheat varieties based on starch type. Apparent energy digestibility ranged from 32 to 63%, with waxy barley varieties having higher energy digestibility coefficients than normal starch varieties. Waxy starch varieties had higher starch digestibility in both barley and wheat because of the greater digestibility of amylopectin than amylose. In summary, the higher energy digestibility of waxy barley lines suggests that these varieties warrant further attention as feed ingredients for rainbow trout.

KEY WORDS: barley, digestibility, starch, trout, wheat

Received 20 December 2007, accepted 16 April 2008

Correspondence: T. Gibson Gaylord, USDA/ARS/PWA Hagerman Fish Culture Experiment Station, 3059-F National Fish Hatchery Road, Hagerman, ID 83332, USA. E-mail: Gibson.Gaylord@ars.usda.gov

Introduction

Cereal grains are included in extruded diets for rainbow trout primarily for their starch component, which is a source of energy and aids in pellet binding and, because of its expansion upon extrusion, allows the pellet to float. Floating feed facilitates management by allowing visualization and regulation of feeding activity. Extrusion processing also increases the digestibility of the starch in cereal grains, therefore, increasing the available energy from the grain. Wheat (*Triticum aestivum* L.) is the primary cereal grain used in extruded diets for trout, however, barley (*Hordeum vulgare* L.) has shown promise as an alternative because of local availability, higher levels of β -glucan, and the recent development of low phytic acid (LPA) varieties (Sealey *et al.* in press; Overturf *et al.* 2003).

Barley utilization in fish feeds is limited because of factors that make processing difficult or lower the nutritional value of the ingredient relative to more commonly used ingredients such as wheat, wheat flour, wheat midds, or mill run. The presence of hulls on standard barley varieties, for example, makes barley difficult to grind with normal hammer mills and impedes extrusion through the die if a small enough particle size is not achieved. The nutritional value of grain hulls to fish is virtually nil, thus they dilute the value of the remaining nutritional components of barley. Additionally, because of low protein content, barley has been included primarily for its starch value. Trout do not utilize starch effectively as an energy substrate relative to protein or fat; however, extrusion processing does improve its digestible energy value through starch gelatinization (Cheng & Hardy 2003). A further disadvantage of barley, as well as other grains, is that the

phosphorus is bound primarily as phytate that cannot be digested effectively by trout and thus adds to the P waste load (Sugiura *et al.* 1999; Cheng & Hardy 2002; Overturf *et al.* 2003). In addition, several factors need to be addressed relative to the nutritional value of barley starch to fish. One factor of importance is the effect of starch type on the nutritional and processing value to fish feeds. Differences in amylose:amylopectin ratios have been shown to differentially affect processing properties in the food science industries (Epstein *et al.* 2002) as well as glucose absorption in both mammals (Zhou & Kaplan 1997) and, to a limited extent, in fish (Rawles & Lochmann 2003).

Since maintaining nutritional and energy balance in extruded fish feeds is paramount to production efficiency, a necessary precursor is the determination of apparent digestibility coefficients (ADC) for nutrients and energy in alternative ingredients. Therefore, the goal of this study was to determine the apparent digestibility of protein, amylose, amylopectin and energy from barley varieties specially bred for improved nutritional value.

Materials and methods

Ingredients and diets

A diverse group of six barley lines with combinations of six selected attributes (normal versus LPA, normal versus waxy, and hulled versus hull-less) were chosen for evaluation in the current study along with a waxy wheat (Morris & King 2007) and soft white wheat varieties (Table 1). The methods of Cho *et al.* (1982) and Bureau *et al.* (1999) were employed to measure *in vivo* apparent digestibility of nutrients. Yttrium oxide served as the inert marker. In brief, a complete reference diet (Table 2) was formulated to meet or exceed all known nutritional requirements of rainbow trout with the exception of phosphorus (NRC 1993). The reference diet was

Table 1 Physical and nutritional characterizations of barley and wheat lines included in extruded diets for rainbow trout

Ingredient	Hull	Phytate	Starch
Barley, Harrington	Hulled	Normal	Normal
Barley, Baronesse	Hulled	Normal	Normal
Barley, Clearwater	Hull-less	LPA ¹	Normal
Barley, 03HR4211	Hull-less	Normal	Normal
Barley, Waxbar	Hull-less	Normal	Waxy
Barley, Merlin	Hull-less	Normal	Waxy
Wheat, Waxy-Pen	Hull-less	Normal	Waxy
Wheat, Soft White	Hull-less	Normal	Normal

¹ LPA, low phytic acid.

formulated to minimize total phosphorus content of the diet to facilitate more accurate measurement of phosphorus availability. Test diets consisted of a 70 : 30 ratio (dry weight basis) of reference diet to test ingredient.

All diets were manufactured using a twin-screw cooking extruder (DNDL-44, Buhler AG, Uzwil, Switzerland). Diet mash was exposed to an average of 127 °C for 18-s in the six extruder barrel sections. The die plate was water cooled to an average temperature of 60°C. Pressure at the die head varied from 200 to 320 psi depending on test ingredient. The 3.0-mm pellets were then dried in a pulse bed drier (Buhler AG) for 25 min at 102 °C with a 10 min cooling period and resulted in final moisture levels of <10%. All oil was included in the mix, rather than top-coated.

Fish and sample collection

Rainbow trout (*Oncorhynchus mykiss*), Housecreek strain, were obtained from the College of Southern Idaho (Twin Falls, ID, USA) and stocked at a rate of 50, 250 g fish per 140-L fibre glass tank. Water temperature was maintained at 15 °C using flow-through spring water and lighting was provided on a 14 : 10 h diurnal cycle. Each diet was randomly assigned to a tank of fish and diets were fed in triplicate by replicating over time as described below. Fish

Table 2 Composition (dry weight basis) of the reference diet fed to rainbow trout

Ingredient	g kg ⁻¹
Corn gluten meal ¹	345.7
Wheat gluten meal ¹	70.4
Soybean meal, solvent extracted dehulled ¹	189.6
Wheat flour ¹	223.2
Lysine-HCl ²	14.7
Taurine ²	5.0
Fish oil ¹	134.3
Stay C 35 ³	3.0
Choline CL, 35 ¹	5.0
Vitamin premix #30 ¹	8.0
Trace mineral premix #3 ¹	1.0
Yttrium oxide ⁴	0.1
Analysed composition: ⁵	
Crude protein (g kg ⁻¹)	463.5
Phosphorus (g kg ⁻¹)	4.6
Gross energy (MJ kg ⁻¹)	23.2

¹ Nelson & Sons Inc., Murray, UT, USA.

² Archer Daniels Midland Company, Decatur, IL, USA.

³ Vitamin C as Rovimix® Stay-C® 35, DSM Nutritional Products, Basel, Switzerland.

⁴ Sigma-Aldrich Company, St. Louis, MO, USA.

⁵ Dry weight basis.

were fed their respective diets to apparent satiation twice daily for 7 days prior to faecal collection. Faecal samples were obtained in one collection by manual stripping 16–18 h postprandial. Manual stripping was accomplished by netting and anaesthetizing all fish in the tank, followed by gently drying and then applying pressure to the lower abdominal region to express faecal matter into a plastic weighing pan. Care was taken to exclude urinary excretions from the collection. Subsequently, new fish were placed in the tanks and diets were randomly reassigned to the tanks for the second and third replicate faecal collections from each diet. Faecal samples for a given tank were dried overnight at 50 °C and stored at –20 °C until chemical analyses were performed.

Chemical analysis

Dry matter and ash analysis of ingredients, diets and faeces were performed according to standard methods (AOAC 1995). Organic matter was calculated as 100-ash content. Yttrium and phosphorus were determined in diets and faeces by an independent laboratory (University of Idaho Analytical Laboratory Services, Moscow, ID, USA) using inductively coupled plasma atomic absorption spectrophotometry. Crude protein ($N \times 6.25$) was determined by the Dumas method (AOAC 1995) on a Leco TruSpec N nitrogen determinator (LECO Corporation, St. Joseph, MI, USA). Total energy was determined by adiabatic bomb calorimetry (Parr6300, Parr Instrument Company Inc., Moline, IL, USA).

Starch was measured according to an enzymatic method using a starch test kit (R-Biopharm, Inc., Marshall, MI, USA). Briefly, samples were treated with dimethylsulfoxide and HCl to solubilize starch. Starch was then hydrolysed to D-glucose in the presence of amyloglucosidase and then reacted with hexokinase and glucose-6-phosphate dehydrogenase to produce NADPH. The amount of NADPH (reduced nicotinamide-adenine dinucleotide phosphate) formed in the reaction is stoichiometrically related to the amount of D-glucose from starch and was determined colorimetrically.

Amylose content of starch was determined by adapting the method of Hovenkamp-Hermelink *et al.* (1988). Starch was extracted with 2 M NaOH and neutralized with 1 M HCl. Upon dilution with water, 2 mL of I₂-KI solution (0.03%:1.5%) was added to 0.5 mL of the starch slurry. After 10 min, the absorbance at 620 and 535 nm was measured on a spectrophotometer. Amylose concentration was then calculated from the ratio of A₆₂₀/A₅₃₅ based on a

standard curve of starch solutions having known concentrations of amylose. Amylopectin concentration was calculated by 100% amylose in starch.

Apparent digestibility coefficients of nutrients in the test diet and ingredients were calculated according to the following equations (Kleiber 1961; Forster 1999):

$$\text{ADCN}_{\text{diet}} = 100 - 100 \{ \% Y \text{ in diet } X \% \text{ nutrient in feces} \} / \{ \% Y \text{ in feces } X \% \text{ nutrient in diet} \} \text{ADCN}_{\text{ingredient}} \\ = \{ (a + b) \text{ADCN}_t - (a) \text{ADCN}_r \} b^{-1}$$

where $\text{ADCN}_{\text{ingredient}}$ is the apparent digestibility coefficient of the nutrient in the test ingredient. ADCN_t is the apparent digestibility coefficients of the nutrient in the test diets. ADCN_r is the apparent digestibility coefficients of the nutrient in the reference diet. $a = (1-p) \times$ nutrient content of the reference diet. $b = p \times$ nutrient content of the test ingredient. P is the proportion of test ingredient in the test diet.

Statistical analysis

The software program Proc GLM of sas version 9.1 (SAS Institute Inc., Cary, NC, USA) was used to conduct a factorial analysis of variance of the effect of cereal type, starch type or their interaction on the digestibility of nutrients and energy in each of the test ingredients. Tukey's means separation was used to ascertain differences among ADC (Tukey 1953; Kramer 1956). When significant interactions were observed, analysis of variance was performed within a cereal type to determine the differences in digestibility coefficients between starch types (normal versus waxy). Single-factor analysis of variance was performed across barley lines to determine the influence of hulled versus hull-less varieties on apparent digestible energy and to determine the effect of the LPA mutation on available phosphorus coefficients. Effects were considered significant at $P \leq 0.05$.

Results

Proximate compositional analyses for the barley and wheat lines are reported in Table 3. Cereal type (barley versus wheat) had no effect on phosphorus, protein, energy or starch ADC as a main effect (Table 4). Effects were noted for cereal type on amylose and amylopectin digestibility, with barley having higher amylose digestibility and wheat having higher amylopectin digestibility. Differences in starch type (waxy versus normal) affected protein, starch, amylose, and amylopectin digestibility. Interactions were observed between

Table 3 Composition of test ingredients in extruded diets for rainbow trout

Ingredient	DM ¹	Phosphorus	Phytate-P ²	Ash	OM ²	CP ²	Energy ²	Starch	Amylose	Amylopectin
Barley, Harrington	924.	4.1	1.9	25.3	975	158	18.4	532	149	383
Barley, Baroness	928.	3.2	1.5	25.8	974	144	18.3	520	147	373
Barley, Clearwater	925.	3.5	1.1	19.8	980	125	18.2	594	168	426
Barley, 03HR4211	924.	3.8	1.8	18.7	981	159	18.5	577	151	426
Barley, Waxbar	928.	3.8	2.0	20.7	979	181	18.5	504	21	483
Barley, Merlin	915.	4.3	2.0	22.3	978	158	18.3	565	31	534
Wheat, Waxy	948.	4.3	2.2	18.5	981	165	18.1	572	32	540
Wheat, Soft White	911.	4.2	2.4	18.6	981	152	18.2	607	125	482

¹ DM = dry matter (g kg⁻¹wet weight) all other compositional analyses are reported on a dry weight basis.

² Phytate-P, phytate phosphorus; OM, organic matter; CP, crude protein.

³ MJ kg⁻¹ dry weight.

Table 4 Apparent digestibility coefficients of phosphorus, crude protein, gross energy, starch, amylose and amylopectin in barley and wheat varieties included in extruded diets for rainbow trout¹

Ingredient	P	CP ²	Energy	Starch	Amylose	Amylopectin
Barley, Harrington	37	94	34	41	8	40
Barley, Baroness	75	125	45	47	27	46
Barley, Clearwater	78	84	35	49	28	47
Barley, 03HR4211	50	91	51	53	16	55
Barley, Waxbar	56	81	41	54	-81	53
Barley, Merlin	54	91	63	77	-75	81
Wheat, waxy	69	53	32	66	-168	68
Wheat, soft white	17	112	46	51	-18	61
Pooled SEM	6.2	8.9	4.3	3.9	25.1	6.0
Analysis of variance						
Pr > F ³	0.004	0.004	0.003	0.003	<0.001	0.002
Cereal	0.070	0.192	0.449	0.297	<0.001	0.035
					wheat < barley	wheat > barley
Starch	0.081	0.005	0.232	<0.001	<0.001	0.001
				n < w	n > w	n < w
Interaction ⁴	0.002	0.017	0.032	0.699	0.175	0.319
Barley	n = w	n = w	n < w			
Wheat	n < w	n > w	n = w			

¹ Values are means of N = 3 replicate tanks of fish per test ingredient.

² Crude protein.

³ Probability associated with the F-statistic.

⁴ If a significant interaction occurred between main effects then differences between starch types (w = waxy versus n = normal) within cereal type were determined.

cereal and starch types for phosphorus availability, as well as protein and energy digestibility.

Interactions between starch type and cereal type were observed in apparent P availability (Table 4). Within barley lines, starch type had no effect on P availability; however, waxy wheat had higher P availability than normal, soft-white wheat. Although not statistically different, there was a trend ($P = 0.07$) toward higher phosphorus availability (78%) in the LPA mutation of 'Clearwater' (Bregitzer *et al.* 2007) barley when compared to an average phosphorus ADC of 55% for all other barleys.

Interaction of cereal and starch types also was observed in the apparent digestibility of protein and energy. Soft white

wheat had higher protein ADC than waxy wheat, whereas, no effects of starch type were observed among the tested barley lines. Waxy barleys had higher digestible energy coefficients than normal barleys, while no effects of starch type were observed in apparent digestible energy coefficients between wheat varieties. Hulls had no effect on digestible energy coefficients within barley varieties. Hulled varieties had an average energy ADC of 39% compared to 47% ($P = 0.22$) for hull-less varieties.

Apparent starch digestibility coefficients were different between starch types but not among cereal types, and there was no interaction. Apparent digestibility coefficient of starch was 65% for waxy varieties, versus 48% for the

normal starch varieties. Apparent amylose digestibility was affected by cereal type and starch type with no interaction. Normal starch type barleys and wheat had higher amylose digestibility coefficients than waxy varieties. Barley also had higher amylose digestibility than wheat. Apparent amylopectin digestibility was different among cereal types and starch types, with no interactions. Both wheat lines had higher average amylopectin digestibility than the barley varieties tested. Waxy varieties had higher apparent amylopectin digestibility than the normal starch type varieties.

Discussion

Considerable phenotypic variability exists in barley lines that may recommend them for specific selection of improvements in nutritional quality for aquafeeds, depending on the species of fish and the barley variety (Rudi *et al.* 2006). A wealth of information is accumulating with respect to the nutritional value of different barley lines for broiler chickens, swine and cows; however, very little information is currently available for fish. Experiments with LPA barley lines show promise in increasing the apparent phosphorus availability for rainbow trout (Sugiura *et al.* 1999; Overturf *et al.* 2003). In the current experiment, values obtained for P availability from 'Harrington' (Harvey & Rossnagel 1984) barley were lower than values obtained by Overturf *et al.* (2003) at 39 versus 52%. The P availability value obtained for Clearwater barley (78%) was comparable to that of the M635 mutant (77%) (Overturf *et al.* 2003). The high P availability observed in 'Baronesse' was unexpected and difficult to explain.

Also counterintuitive is the difference in P availability between wheat starch types: P availability was higher in waxy wheat compared to the normal starch soft white wheat. Yamamoto *et al.* (2007) noted that P absorption from diets for common carp (*Cyprinus carpio*) was lower when gelatinized starch was included when compared to diets that included raw starch; however, the mechanism by which this occurred is unresolved. Potential mechanisms by which starch type may influence P availability are the development of resistant starch in amylose as well as phosphorylation of starch by heat and moisture during the feed extrusion process. The extent to which this can occur is not well characterized, although Sang & Seib (2006) examined the development of resistant starches in high-amylose corn mutants when a heat-moisture treatment and phosphorylation were applied. Further characterization of this potential effect will need to be addressed in future research.

One of the most prominent phenotypic variations in barley nutritional quality for animals is the ratio of starch fractions.

Several cereal grains have been developed with altered amylose:amylopectin ratios for various processing and nutritional purposes. Pettersson and Lindberg (1997) tested the potential variation in ileal and total tract digestibility of barleys in pigs when hull content and starch type (amylose:amylopectin ratio) varied. They observed improved organic matter and energy digestibility with naked versus hulled types and a small improvement in ileal starch digestibility in the high-amylopectin, hulled line versus the high-amylose hulled line. Lindberg *et al.* (2003) also observed improvements in total tract digestibility of organic matter from hull-less barley varieties with high amylopectin content.

Limited data are available on the digestibility of different starch fractions for fish. Enes *et al.* (2006) compared the digestibility of waxy maize to normal starch maize in European sea bass (*Dicentrarchus labrax*) and found that waxy starch was more digestible at 20% inclusion levels than normal starch, but not at 10% inclusion levels. They also observed notably higher starch digestibility in sea bass than what we observed in the current trial, even though their diets were cold pelleted. Cheng & Hardy (2003) observed positive effects of extrusion processing on energy digestibility from barley and wheat, most likely because of increased gelatinization of the starch fraction of the cereal grains. However, contrary to the current study, the digestibilities of the individual starch fractions were not reported by Enes *et al.* (2006).

In the current trial, crude starch digestibility was improved in the waxy versus normal starch lines in both cereal grains, similar to that observed for corn starches in European sea bass as reported by Enes *et al.* (2006). This was further confirmed in rainbow trout by analysing both amylose and amylopectin digestibilities in the present trial. The highly negative amylose digestibility is rather striking in the waxy lines and was most likely because of the low amylose content that is characteristic of waxy cereal varieties and is often difficult to measure with current digestibility methodology. Nevertheless, it is clear from the data that amylose digestibility is reduced compared to amylopectin, even among normal starch type varieties.

Significant interactions occurred in the apparent digestibility of energy in the grains tested. Interestingly, no differences were discernable for waxy versus normal wheat; however, differences were observed between starch types within barley lines. The waxy barleys had higher digestible energy coefficients than the normal barleys, which corroborates the improved amylopectin digestibility when compared to amylose. One confounding factor was the hull inclusion in two lines of barley with normal starch types. The hull would potentially decrease the apparent digestible energy from these

line of barley, but when statistical analyses were performed for hull effects on digestible energy in normal starch barley lines, no differences were observed ($P = 0.77$).

Conclusions

The ability to utilize varieties of cereal grains selected for improved nutritional value has been limited in rainbow trout feeds because of insufficient characterization of the impacts on fish production parameters. This study is a first step in defining the nutritional value of the selected grains. Further characterization of the nutrient affects on feed quality, as well fish growth rates and efficiency is also needed. Observations from the current trial indicate that, because of their higher amylopectin content, waxy barleys will have a higher digestible energy value for rainbow trout than normal starch barleys. However, the significance of the increased energy availability from carbohydrates also will need to be addressed, as trout use carbohydrates less efficiently as an energy yielding substrate than protein/amino acids or fats. Therefore, regression estimates of the available energy from differing starch types at differing levels of inclusion will be necessary to balance available energy in trout diets.

Acknowledgements

We wish to thank ARS technicians April M. Teague, Rebecca Jacobs, Lorrie Van Tassel, Mike Woolman, ARS SCEP student Scott Snyder and University of Idaho personnel Mike Casten and Lucas Porter for their assistance with this study. This study was funded by the USDA/Agricultural Research Service Project Numbers 5366-21310-003-00D.

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